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Two New Species of *Dicyema* (Dicyemida: Dicyemidae) from *Amphioctopus areolatus* (Mollusca: Cephalopoda: Octopodidae)

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Two new species of dicyemid mesozoan are described from *Amphioctopus areolatus* (de Haan, 1840) collected off Irino in Tosa Bay, southern Shikoku, and off Akashi in Osaka Bay, Honshu, Japan. *Dicyema balanocephalum* sp. nov. is a species of medium size that reaches about 1500 μm in length. The vermiform stages are characterized by 19–21 peripheral cells, an elongate calotte, and an axial cell that extends to the base of the propolar cells. Infusoriform embryos consist of 37 cells; two nuclei are present in each urn cell, and the refringent bodies are solid. *Dicyema leiocephalum* sp. nov. is a species of medium size that reaches about 2000 μm in length. The vermiform stages are characterized by 22 peripheral cells, a conical, smooth-surfaced calotte, and an axial cell that extends to the base of the propolar cells. Infusoriform embryos consist of 37 cells; two nuclei are present in each urn cell, and the refringent bodies are solid. This is the first report of dicyemids in *A. areolatus*.

Key Words: cephalopods, dicyemids, infusoriform embryos, mesozoans, parasites, renal organs, *Amphioctopus areolatus*, vermiform embryos.

Introduction

In Japan the first record of dicyemids was published by Nouvel and Nakao (1938). They described *Dicyema misakiense* Nouvel and Nakao, 1938 from *Octopus vulgaris* Lamarck, 1798, and *D. orientale* Nouvel and Nakao, 1938 from *Sepioteuthis lessoniana* Lesson, 1830. Nouvel (1947) later described *D. acuticephalum* Nouvel, 1947 from *O. vulgaris* and identified a dicyemid from *Sepia esculenta* Hoyle, 1885 as *Pseudicyema truncatum* Whitman, 1883, which had been described earlier in Europe. Subsequently, two dicyemids, *D. japonicum* Furuya and Tsuneki, 1992 and *D. clavatum* Furuya and Koshida, 1992, were described from *O. vulgaris* and *Callistoctopus minor* (Sasaki, 1920), respectively (Furuya *et al.* 1992a). Furuya (1999) later reported 14 new species of dicyemid from six cephalopod species caught off the coasts of Japan: *Amphioctopus fangsiao* (d'Orbigny, 1840), *Callistoctopus minor*, *Octopus hongkongensis* Hoyle, 1885, *Enteroctopus dofleini* (Wülker, 1910), *Sepia esculenta*, and *S. lycidas* Gray, 1849. More recently, a new dicyemid was described from *Sepioteuthis lessoniana* by Furuya and Tsuneki (2005), three new dicyemids from *Amphioctopus kagoshimensis* (Ortmann, 1888) by Furuya (2005), and three new di-

cyemids from *A. fangsiao* by Furuya (2006).

In this paper two new species in the genus *Dicyema* are described from *Amphioctopus areolatus* (de Haan, 1840) collected off Irino in Tosa Bay, Shikoku, and off Akashi in Osaka Bay, Honshu. These two species, the existence of which was first noted by Furuya and Tsuneki (2003), are the first dicyemids to be described from *A. areolatus*.

Materials and Methods

Forty-eight individuals of *Amphioctopus areolatus* obtained from fishermen were examined for dicyemids from March, 2001, to January, 2005. The size, sex, and locality of each octopus are indicated in Table 1.

When dicyemids were detected in the kidney of a host cephalopod, small pieces of renal appendages with attached dicyemids were removed and smeared on glass slides. The smears were fixed immediately in Bouin's fluid for 24 hr and then stored in 70% ethyl alcohol before staining. Most slides were stained in Ehrlich's hematoxylin and counterstained in eosin. Stained smears were mounted using Entellan (Merck). Dicyemids were observed with a light microscope (Olympus BH-2) at magnifications up to 2000×. Measurements and drawings were made with the aid of an ocular micrometer and a drawing tube (Olympus U-DA).

Nouvel (1948), Short and Damian (1966), Furuya *et al.* (1992b, 1997), and Furuya (1999) give the terminology for cell names used in the descriptions of infusoriform larvae.

Syntypes of the dicyemids are deposited in the Osaka University Museum, Toyonaka, Osaka, Japan (OUM), the Santa Barbara Museum of Natural History, Santa Barbara, California, USA (SBMNH), and in the author's collection. The host octopuses of the syntypes of the two new dicyemid species are deposited in the OUM.

Abbreviations in figures: A, apical cell; AG, agamete (axoblast); AI, apical internal cell; AL, apical internal cell; AX, axial cell; C, couvercle cell; CA, capsule cell; CL, calotte; D, diapolar cell; DC, dorsal caudal cell; DI, dorsal internal cell; DV, developing vermiform embryo; E, enveloping cell; G, germinal cell; I, infusoriform embryo; L, lateral cell; LC, lateral caudal cell; M, metapolar cell; MD, median dorsal cell; NI, nucleus of infusorigen; O, oogonium; P, propolar cell; PA, parapolar cell; PD, paired dorsal cell; PN, paranucleus; PO, primary oocyte; PVL, posteroventral lateral cell; R, refringent body; S, spermatogonium; SP, sperm; U, urn cell; UC, urn cavity; UP, uropolar cells; VC, ventral caudal cell; VI, ventral internal cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell.

Taxonomy

Dicyema balanocephalum sp. nov.

(Figs 1, 2, Tables 1, 2)

Diagnosis. Medium-sized dicyemids, body lengths typically not exceeding 1500 μm. Peripheral cell number of vermiform stages (i.e., vermiform embryo, ne-

Table 1. Dicyemid species from the octopus *Amphioctopus areolatus*.

Host no.	ML[1] (mm)	Sex[2]	Locality[3]	Date of examination	Dicyemids
AR687	55	♀	1	07 Jan. 2001	*D. balanocephalum*+*D. leiocephalum*
AR688	45	♀	1	07 Jan. 2001	None
AR701	48	♀	1	02 Apr. 2001	*D. balanocephalum*
AR702	52	♀	1	02 Apr. 2001	None
AR703	47	♀	1	02 Apr. 2001	*D. balanocephalum*
AR704	50	♀	1	02 Apr. 2001	None
AR705	46	♀	1	02 Apr. 2001	*D. balanocephalum*
AR706	48	♀	1	02 Apr. 2001	*D. balanocephalum*
AR707	55	♂	1	02 Apr. 2001	*D. balanocephalum*+*D. leiocephalum*
AR708	53	♀	1	02 Apr. 2001	*D. balanocephalum*
AR709	38	♀	1	02 Apr. 2001	*D. balanocephalum*
AR710	55	♀	1	02 Apr. 2001	None
AR711	56	♀	1	02 Apr. 2001	None
AR712	44	♂	1	02 Apr. 2001	*D. leiocephalum*
AR713	45	♀	1	02 Apr. 2001	*D. balanocephalum*
AR714	57	♀	1	02 Apr. 2001	None
AR715	50	♀	1	02 Apr. 2001	*D. leiocephalum*
AR716	60	♀	1	02 Apr. 2001	None
AR717	42	♀	1	02 Apr. 2001	*D. leiocephalum*
AR718	46	♀	1	02 Apr. 2001	*D. balanocephalum*+*D. leiocephalum*
AR760	65	♀	1	03 Apr. 2001	*D. balanocephalum*+*D. leiocephalum*
AR761	62	♀	1	03 Apr. 2001	*D. balanocephalum*
AR762[a]	43	♂	1	03 Apr. 2001	*D. leiocephalum*
AR763	42	♂	1	03 Apr. 2001	*D. leiocephalum*
AR764[b]	50	♀	1	03 Apr. 2001	*D. balanocephalum*
AR765	38	♂	1	03 Apr. 2001	*D. leiocephalum*
AR766	40	♀	1	03 Apr. 2001	*D. leiocephalum*
AR767	51	♂	1	03 Apr. 2001	*D. leiocephalum*
AR768	45	♂	1	03 Apr. 2001	None
AR769	45	♂	1	03 Apr. 2001	*D. leiocephalum*
AR770	38	♂	1	03 Apr. 2001	*D. leiocephalum*
AR771	36	♂	1	03 Apr. 2001	*D. leiocephalum*
AR772	54	♀	1	03 Apr. 2001	*D. leiocephalum*
AR850	46	♂	2	14 Feb. 2003	*D. balanocephalum*
AR851	45	♀	2	14 Feb. 2003	*D. leiocephalum*
AR1130	47	♂	2	14 Jan. 2005	*D. leiocephalum*
AR1131	48	♂	2	14 Jan. 2005	*D. leiocephalum*
AR1132	40	♂	2	14 Jan. 2005	None
AR1133	43	♂	2	14 Jan. 2005	None
AR1134	41	♀	2	14 Jan. 2005	None
AR1135	37	♂	2	14 Jan. 2005	None
AR1136	38	♂	2	14 Jan. 2005	None
AR1137	35	♀	2	14 Jan. 2005	*D. leiocephalum*
AR1188	41	♂	2	21 Jan. 2005	None
AR1189	42	♂	2	21 Jan. 2005	None
AR1267	40	♂	2	26 Jan. 2005	None
AR1268	39	♂	2	26 Jan. 2005	None
AR1269	38	♂	2	26 Jan. 2005	None

[1] Dorsal mantle length.
[2] All specimens were mature.
[3] 1, Tosa Bay (Shikoku); 2, Osaka Bay (Honshu).
[a] The host (symbiotype) of the syntypes of *D. leiocephalum*.
[b] The host (symbiotype) of the syntypes of *D. balanocephalum*.

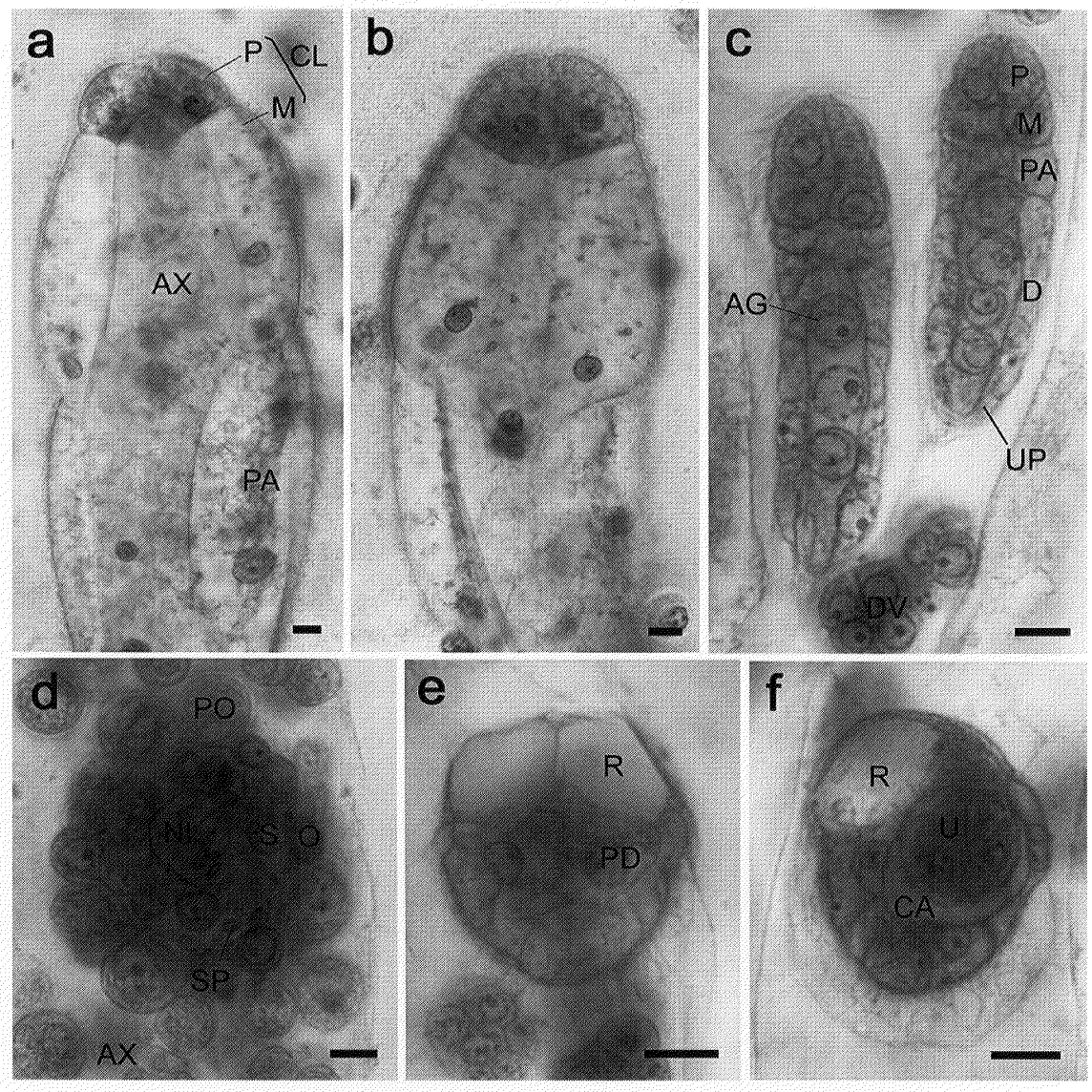

Fig. 1. *Dicyema balanocephalum* sp. nov., syntype specimens on slide OUM-ME-00009. a, Nematogen, anterior region; b, rhombogen, anterior region; c, vermiform embryos within axial cell; d, infusorigen; e, f, infusoriform embryos, horizontal section (e) and sagittal section (f). Scale bars: 5 μm. Abbreviations as in "Materials and Methods".

matogen, and rhombogen) 19–21, i.e., 4 propolars, 4 metapolars, and 11–13 trunk cells. Calotte relatively large, elongate. Infusoriform embryos consisting of 37 cells; urn cells with 2 nuclei each.

Description. *Nematogens* (Figs 1a, 2a, c). Body slender; lengths ranging from 700 to 1500 μm, widths from 60 to 80 μm. Peripheral cell number 19–21, i.e., 4 propolars, 4 metapolars, 2 parapolars, 7–9 diapolars, and 2 uropolars. Calotte bluntly rounded, elongate (Figs 1a, 2c). Cilia on calotte short, about 3 μm long, oriented forwards. Cytoplasm of propolar cells stained with hematoxylin, but metapolar cells not stained (Fig. 1a). Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively. Trunk mostly uniform in width; trunk cells

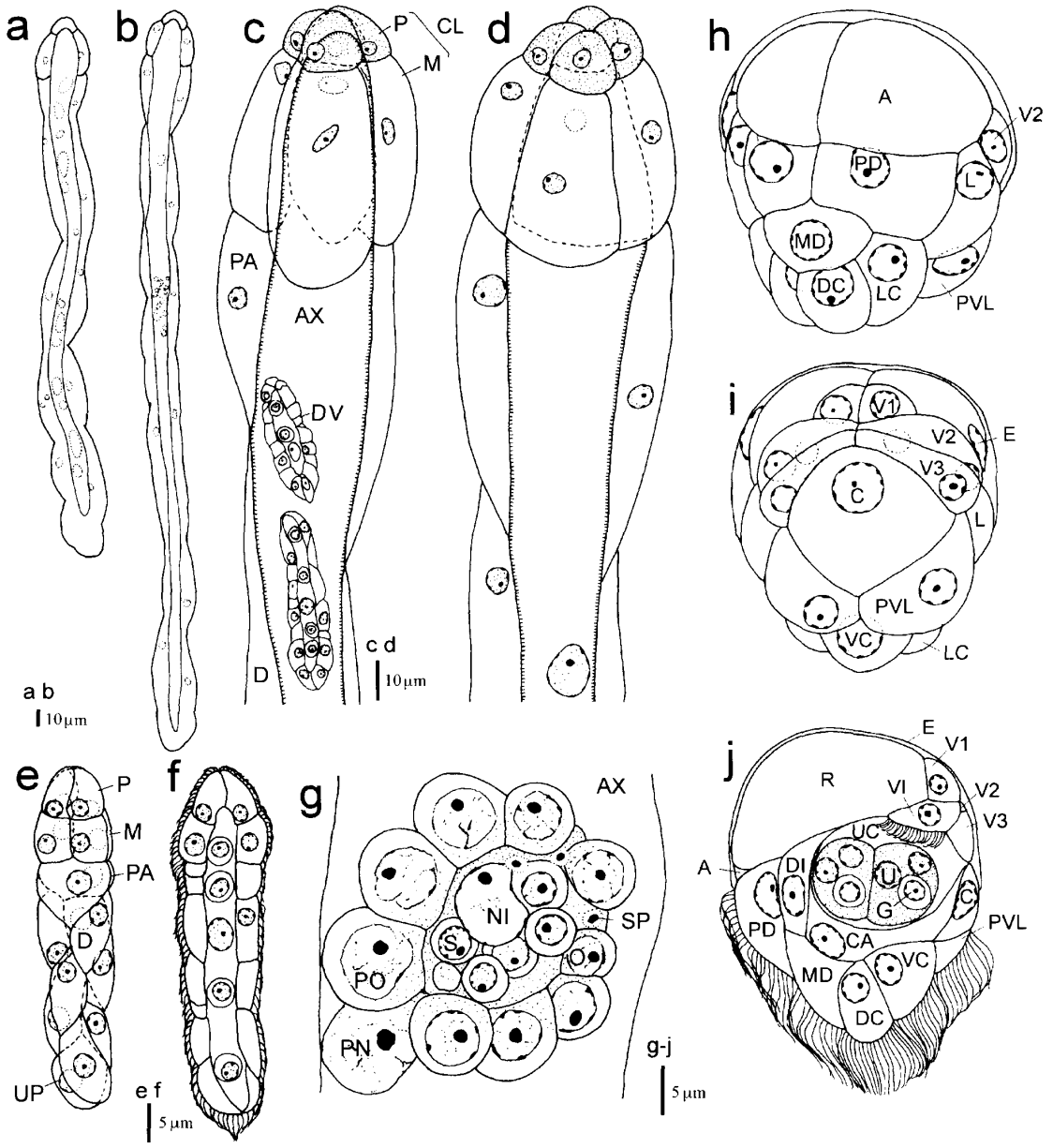

Fig. 2. *Dicyema balanocephalum* sp. nov., syntype specimens on slide OUM-ME-00009. a, Nematogen, entire; b, rhombogen, entire; c, nematogen, anterior region; d, rhombogen, anterior region; e–g, vermiform embryo within axial cell, cilia omitted (e), optical section (f), and infusorigen (g); h–j, infusoriform embryos, dorsal view (h; cilia omitted), ventral view (i; cilia omitted), and sagittal section (j). Abbreviations as in "Materials and Methods".

arranged in opposed pairs. Axial cell cylindrical, rounded anteriorly, extending forward to base of propolar cells (Fig. 1a). In axial cell of large individuals, 20–50 vermiform embryos present. Accessory nuclei occasionally seen in peripheral trunk cells.

Vermiform embryos (Figs 1c, 2e, f). Full-grown vermiform embryos small; lengths ranging from 40 to 60 μm, widths from 10 to 13 μm; peripheral cell numbers 19–21 (Table 2). Anterior end of calotte tapered anteriorly, bluntly rounded at tip.

Trunk cells arranged in opposed pairs. Axial cell rounded anteriorly, extending forward to base of propolar cells. Axial cell nucleus typically located in center of axial cell. Axial cell of full-grown embryos containing 2–5 agametes.

Rhombogens (Figs 1b, 2b, d). Generally similar to nematogens in shape and body proportions; lengths ranging from 700 to 1500 μm, widths from 60 to 80 μm. Peripheral cell numbers 19–21 (Table 2). Calotte elongate, as in nematogens. Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell 1–3; in axial cell of large individuals, 20–40 infusoriform embryos typically present. Accessory nuclei occasionally present in peripheral cells.

Infusorigens (Figs 1d, 2g). Large. Axial cell of infusorigens usually elliptical in shape, ranging from 20 to 30 μm in long diameter. In mature infusorigens (n=20), number of external cells (oogonia and primary oocytes) 38–71 (mode 52), number of internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) 24–45 (mode 34), and number of sperm 12–84 (mode 39). Diameter of fertilized eggs 8.6 μm; diameter of sperm 1.4 μm.

Infusoriform embryos (Figs 1e, f, 2h–j). Ovoid, bluntly pointed posteriorly. In full-grown embryos (n=50), length (excluding cilia) 21.3±1.4 μm (mean±S.D.), length-width-height ratio 1 : 0.83 : 0.80. Cilia at posterior end 6.0 μm long. Refringent bodies present, solid, occupying anterior 40% of embryo length when viewed laterally (Fig. 1f). Cilia of ventral internal cells projecting into urn cavity (Fig. 2j). Cytoplasm of dorsal internal cells transparent. Full-grown infusoriform embryos (n=50) consisting of 37 cells, i.e., 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, and 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, and 1 couvercle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 dorsal internal cells, 2 capsule cells, and 4 urn cells). Each urn cell containing 1 germinal cell plus 2 nuclei (Fig. 2j). Nuclei of second ventral cells pycnotic. All somatic nuclei typically becoming pycnotic as infusoriform embryos mature.

Type series. Syntypes: OUM-ME-00009 (1 slide); SBMNH-359814 (1 slide); No. HF-AR764 (1 slide) (author's collection).

Type locality. Japan, Shikoku, Kochi Prefecture, Tosa Bay, off Irino, 32°55′N, 137°10′E.

Host (symbiotype). *Amphioctopus areolatus* (Cephalopoda: Octopoda: Octopodidae), mature female, 50 mm ML (mantle length), OUM-MO-00008 (AR764 in Table 1).

Site of infection. Within renal sacs; anterior ends (calottes) inserted into crypts of the renal appendages.

Prevalence. Dicyemids found in 14 host cephalopods obtained in Tosa Bay (Irino) and Osaka Bay (Akashi), with 29.2% prevalence among 48 cephalopods examined (see Table 1).

Distribution. Type locality and off Akashi, Osaka Bay, Honshu, Japan.

Etymology. The specific name is an adjective composed of two Greek roots, *balanos*, meaning "acorn", and *-kephalos*, meaning "-headed", in reference to the

Table 2. Number of peripheral cells in two new species of *Dicyema*.

Species	Cell number	Number of individuals		
		Nematogens	Vermiform embryos	Rhombogens
Dicyema balanocephalum	19	8	4	7
	20	37	39	40
	21	5	7	3
Dicyema leiocephalum	21	1	0	3
	22	49	50	47

characteristic acorn-shaped calotte of the adult stages.

Remarks. *Dicyema balanocephalum* is very similar to *D. dolichocephalum* Furuya, 1999, a dicyemid of *Callistoctopus minor*, in having an elongate calotte (Furuya 1999). However, *D. balanocephalum* is distinguishable from *D. dolichocephalum* in the maximum body length of vermiform stages (1500 μm vs. 800 μm), the average length of infusoriform embryos (21.3 μm vs. 28.0 μm), the size of infusorigens (10 μm vs. 15–20 μm in diameter), and the number of peripheral cells (19–21 vs. 18 or 20). *Dicyema balanocephalum* can be distinguished from all the other species in the genus on the basis of its calotte shape, small propolar cells, long metapolar cells, and number of peripheral cells.

In contrast to the distinct calotte shape, the infusoriform embryos of *D. balanocephalum* are of typical type with respect to cellular composition and cell number (Furuya *et al.* 2004).

Dicyema leiocephalum sp. nov.
(Figs 3, 4, Tables 1, 2)

Diagnosis. Medium-sized dicyemids, body lengths typically not exceeding 2000 μm. Peripheral cell number of vermiform stages (i.e., vermiform embryo, nematogen, and rhombogen) 22, i.e., 4 propolars, 4 metapolars, and 14 trunk cells. Calotte relatively large, conical, smooth-surfaced. Infusoriform embryos consisting of 37 cells; urn cells with 2 nuclei each.

Description. *Nematogens* (Figs 3a, 4a, c). Body slender; lengths ranging from 600 to 2000 μm, widths from 30 to 70 μm. Peripheral cell number 22, i.e., 4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, and 2 uropolars. Calotte bluntly rounded, conical, smooth-surfaced (Figs 3a, 4c). Cilia on calotte short, about 3 μm long, oriented forwards. Propolar cells and their nuclei smaller than metapolar cells and their nuclei, respectively (Fig. 3a). Trunk mostly uniform in width; trunk cells arranged in opposed pairs. Axial cell cylindrical, rounded anteriorly, extending forward to base of propolar cells (Fig. 4c). In axial cell of large individuals, 12–50 vermiform embryos present. Accessory nuclei seen in peripheral trunk cells.

Vermiform embryos (Figs 3c, 4e, f). Full-grown vermiform embryos medium-sized; lengths ranging from 70 to 110 μm, widths from 9 to 13 μm; peripheral cell number 22 (Table 2). Anterior end of calotte tapered anteriorly, bluntly rounded at

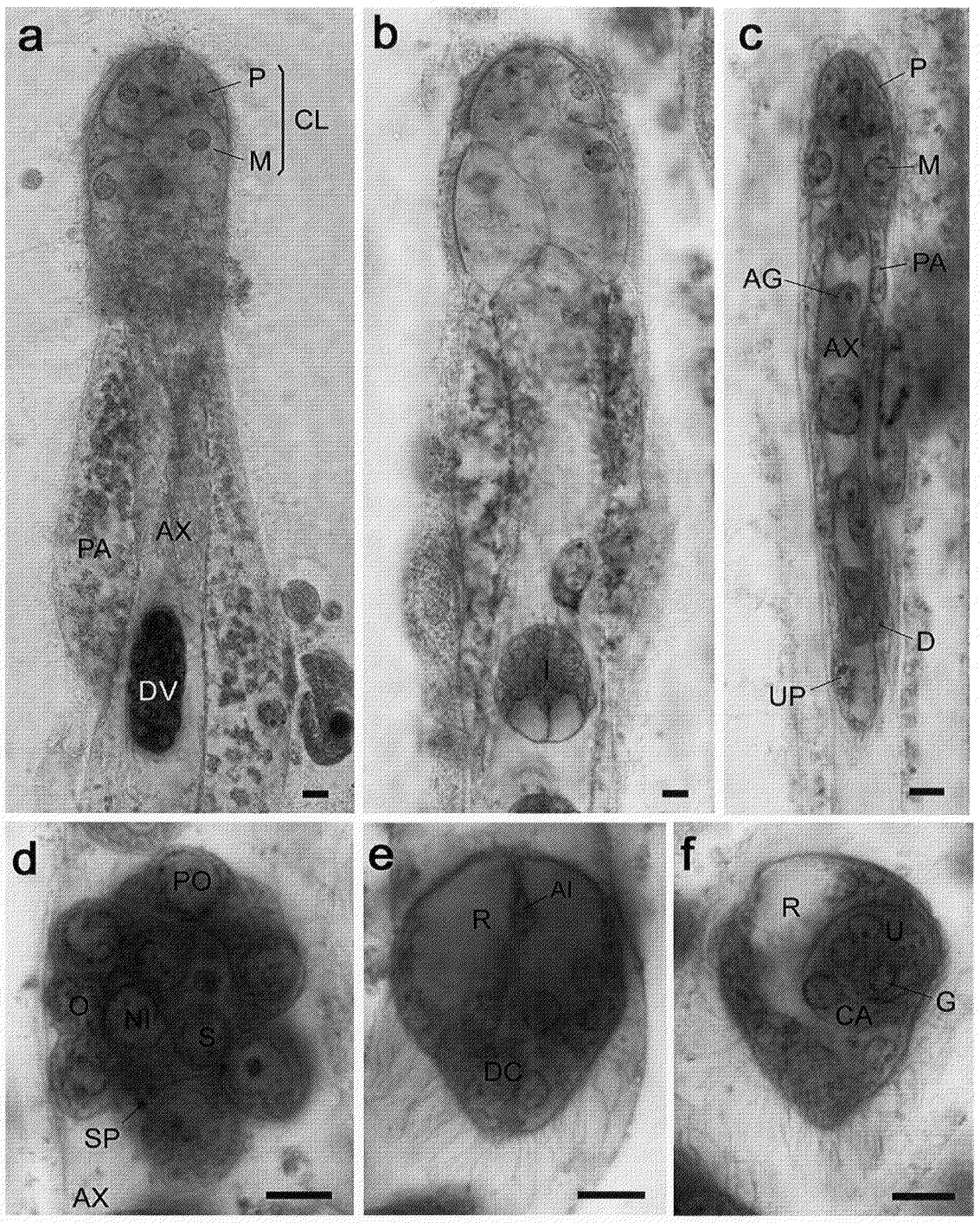

Fig. 3. *Dicyema leiocephalum* sp. nov., syntype specimens on slide OUM-ME-00010. a, Nematogen, anterior region; b, rhombogen, anterior region; c, vermiform embryo within axial cell; d, infusorigen, e, f, infusoriform embryo, horizontal section (e) and sagittal section (f). Scale bars: 5 μm. Abbreviations as in "Materials and Methods".

tip. Cytoplasm of propolar cells stained with hematoxylin, but metapolar cells not stained (Fig. 3c). Trunk cells arranged in opposed pairs. Axial cell rounded anteriorly, extending forward to base of propolar cells. Axial cell nucleus typically lo-

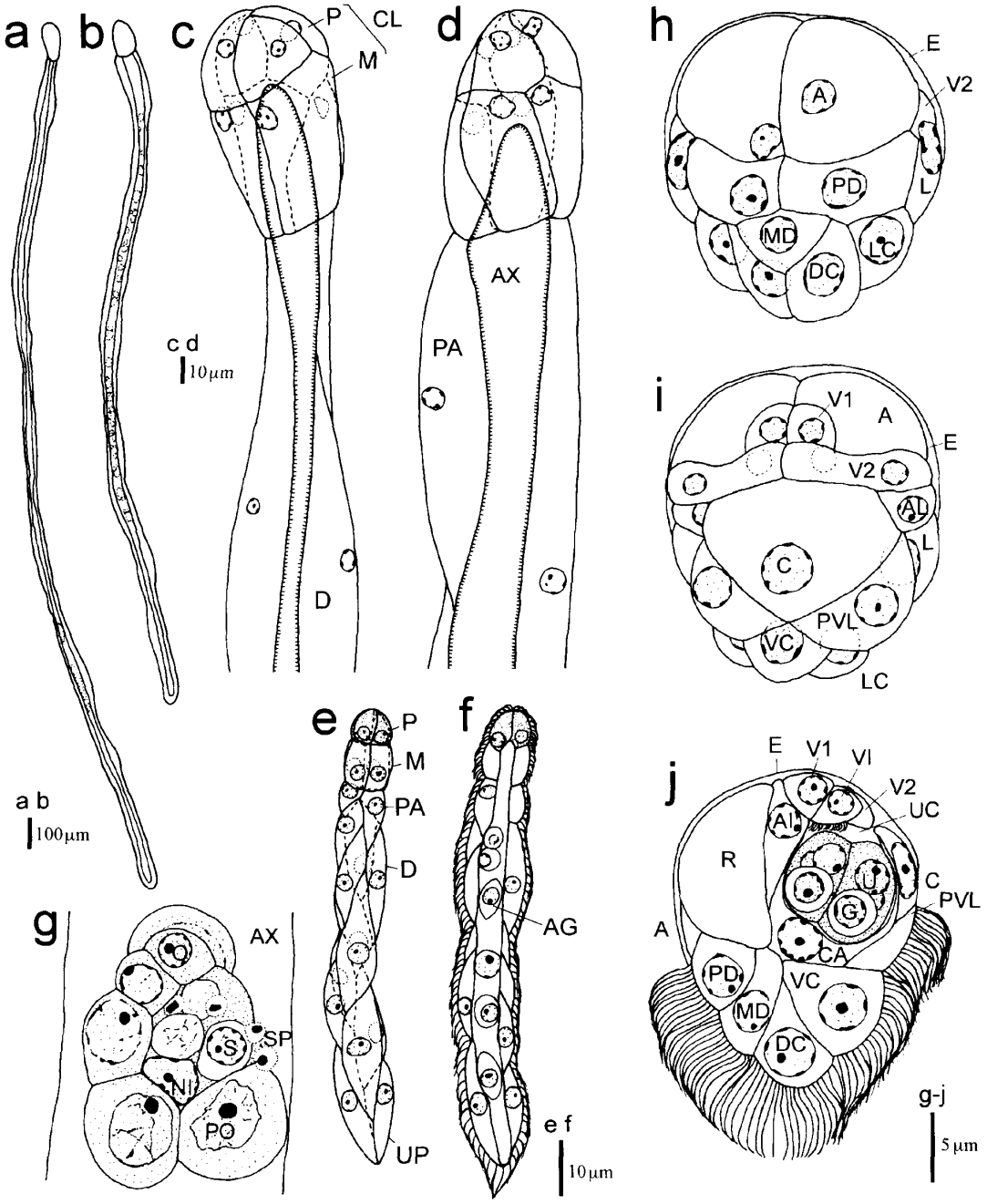

Fig. 4. *Dicyema leiocephalum* sp. nov., syntype specimens on slide OUM-ME-00010. a, Nematogen, entire; b, rhombogen, entire; c, nematogen, anterior region; d, rhombogen, anterior region; e–g, vermiform embryos within axial cell, cilia omitted (e), optical section (f), and infusorigen (g); h–j, infusoriform embryos, dorsal view (h; cilia omitted), ventral view (i; cilia omitted), and sagittal section (j). Abbreviations as in "Materials and Methods".

cated in center of axial cell. Axial cell of full-grown embryos containing 4–11 agametes, some of latter elliptical (Figs 3c, 4f).

Rhombogens (Figs 3b, 4b, d). Slightly stockier than nematogens, otherwise generally similar in shape and body proportions; lengths ranging from 600 to 2000 μm,

widths from 30 to 80 μm. Peripheral cell number typically 22 (Table 2). Calotte conical as in nematogens. Shape and anterior extent of axial cell similar to those of nematogens. Number of infusorigens present in axial cell 1 or 2; in axial cell of large individuals, 10–42 infusoriform embryos typically present. Accessory nuclei present in peripheral cells.

Infusorigens (Figs 3d, 4g). Medium-sized. Axial cell of infusorigens usually rounded, diameter 10–13 μm. In mature infusorigens (n=20), number of external cells (oogonia and primary oocytes) 6–12 (mode 10), number of internal cells (spermatogonia, primary spermatocytes, and secondary spermatocytes) 3–5 (mode 4), and number of sperm 4–6 (mode 5). Diameter of fertilized eggs 9.6 μm; diameter of sperm 1.3 μm.

Infusoriform embryos (Figs 3e, f, 4h–j). Ovoid, bluntly pointed posteriorly. In full-grown embryos (n=50), length (excluding cilia) 22.4±1.6 μm (mean±S.D.), length-width-height ratio 1 : 0.74 : 0.73. Cilia at posterior end 4.8 μm long. Refringent bodies present, solid, occupying anterior 40% of embryo length when viewed laterally (Figs 3f, 4j). Nuclei of apical internal cells usually visible between refringent bodies (Fig. 3e). Cilia of ventral internal cells projecting into urn cavity (Fig. 4j). Cytoplasm of capsule cells transparent. Full-grown infusoriform embryos (n=50) consisting of 37 cells, i.e., 33 somatic and 4 germinal cells. Somatic cells of several types: external cells covering large part of anterior and lateral surfaces of embryo (2 enveloping cells); external cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, and 2 posteroventral lateral cells); external cells with refringent bodies (2 apical cells); external cells without cilia (2 anterior lateral cells, 2 first ventral cells, 2 second ventral cells, and 1 couvercle cell); internal cells with cilia (2 ventral internal cells); and internal cells without cilia (2 apical internal cells, 2 capsule cells, and 4 urn cells). Each urn cell containing 1 germinal cell plus 2 nuclei (Fig. 4j). Nuclei of second ventral cells pycnotic. All somatic nuclei typically becoming pycnotic as infusoriform embryos mature.

Type series. Syntypes: OUM-ME-00010 (1 slide); SBMNH-359815 (1 slide); No. HF-AR762 (1 slide) (author's collection).

Type locality. Japan, Shikoku, Kochi Prefecture, Tosa Bay, off Irino, 32°55′N, 137°10′E.

Host (symbiotype). *Amphioctopus areolatus* (Cephalopoda: Octopoda: Octopodidae), mature male, 43 mm ML (mantle length), OUM-MO-00009 (AR762 in Table 1).

Site of infection. Within renal sacs; anterior ends (calottes) inserted into crypts of the renal appendages.

Prevalence. Dicyemids found in 20 host cephalopods obtained in Tosa Bay (Irino) and Osaka Bay (Akashi), with 41.7% prevalence among 48 cephalopods examined (see Table 1).

Distribution. The type locality and off Akashi, Osaka Bay, Honshu, Japan.

Etymology. The specific name is an adjective composed of two Greek roots, *leios*, meaning "smooth", and *-kephalos*, meaning "-headed", in reference to the characteristic smooth-surfaced calotte of the adult stages.

Remarks. *Dicyema leiocephalum* is characterized by a conical, smooth-surfaced calotte, 22 peripheral cells, and infusoriform embryos lacking dorsal internal cells. In these respects *D. leiocephalum* is easily distinguished from *D. bal-*

anocephalum.

The size and number of infusorigens are diagnostic characteristics of dicyemid species (Furuya *et al.* 1993). There is a negative curvilinear relationship between the number of infusorigens per rhombogen and the number of gametes per infusorigen (Furuya *et al.* 2003b). Two distinct groups of dicyemid species are apparent: (1) those with rhombogens that form a small number of infusorigens that each produces a relatively large number of gametes (up to 70); (2) those with rhombogens that tend to form a large number of infusorigens, each of which produces at most 20 gametes. Rhombogens of *D. leiocephalum* have a small number of medium-sized infusorigens and produce a relatively small number of gametes; thus this species does not belong to either of these two groups. Rhombogens of *D. balanocephalum* have a small number of large-sized infusorigens and produce a relatively large number of gametes; thus this species belongs to the first group.

In cellular composition of the infusoriform embryos, *D. leiocephalum* is of a particular type that possesses anterior internal cells and lacks dorsal internal cells (Furuya *et al.* 1997, 2004). In the genus *Dicyema*, this type of infusoriform embryo has not been reported thus far. In other genera, only infusoriform embryos of *Dicyemodeca deca* (McConnaughey, 1957) lack the dorsal internal cells (Furuya *et al.* 2004).

Discussion

There is considerable taxonomic confusion with regard to the group of small to medium-sized species of *Octopus* living primarily on substrates of sand or mud in tropical and subtropical waters of the Indo-Pacific (Robson 1929; Huffard and Hochberg 2005; Norman and Hochberg in press). This group consists of two subgroups, an ocellate subgroup including *Octopus areolatus* and a non-ocellate subgroup including *Octopus aegina* (Gray, 1849). Huffard and Hochberg (2005) proposed to place these two subgroups in the genus *Amphioctopus*.

Amphioctopus areolatus inhabits inshore waters and is distributed from the Seto Inland Sea, and Tosa Bay, to the Chinese coast. This octopus is characterized by a pair of bluish-gray ocelli on the web between arms II and III, three dark stripes on the head, and two stripes on the dorsal mantle. Several species of dicyemids have been found in species of *Amphioctopus*, such as *A. aegina* from Taiwan (Furuya *et al.* unpublished), *A. kagoshimensis* from Japan (Furuya 2005), *A. burryi* (Voss, 1950) from the Gulf of Mexico (Furuya *et al.* 2002), and *A. fangsiao* from Japan (Furuya 1999, 2006). However, dicyemids were not found in an undescribed *Amphioctopus* from Hawaii (Huffard and Hochberg 2005).

In this study the two new dicyemid species, *Dicyema balanocephalum* and *D. leiocephalum*, were found in 30 of 48 examined individuals of *Amphioctopus areolatus* caught in Tosa Bay and Osaka Bay. This data revealed geographical variations in prevalence, 75.8% (25/33) in Tosa Bay and 33.3% (5/15) in Osaka Bay. There is a direct relationship between host size and dicyemid occurrence (Furuya *et al.* 1992a): smaller or younger cephalopods of a host species generally do not harbor dicyemids. In *A. areolatus*, however, the 18 examined individuals that harbored no dicyemids were medium- and large-sized and the absence of dicyemids in them can not be attributed to host size.

In cephalopods that harbor two or three dicyemid species in the renal sac, the calotte shapes are typically different from each other (Furuya *et al.* 2003a). Dicyemids that have similar calotte shapes are very rarely found together in a single host individual. In *A. areolatus*, the calotte shapes of *D. leiocephalum* and *D. balanocephalum* are conical and elongate, respectively, but both shapes fall into the conical category recognized by Furuya *et al.* (2003a). In accordance with expectations, co-occurrence of the two present dicyemid species was only 13.3% (4/30).

With respect to cellular composition and cell number, the infusoriform embryos of *D. balanocephalum* are of the typical type (Furuya *et al.* 2004). In contrast, *D. leiocephalum* is characterized by infusoriform embryos that possess anterior internal cells and lack dorsal internal cells. Infusoriform embryos possessing anterior internal cells have been found in the dicyemids from *A. fangsiao*, *A. kagoshimensis*, and *Octopus vulgaris* in Japan. These *Amphioctopus* species are closely related to *O. vulgaris* (Takumiya *et al.* 2005). I speculate possession of anterior internal cells by infusoriform embryos of dicyemids in these host species may be attributed to the close relationships among the host species.

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